How do you set up the Purity Thresholds in Empower 3? - WKB11215

OBJECTIVE or GOAL

To establish Purity Thresholds.

ENVIRONMENT

- Empower 3
- PDA data

PROCEDURE

Use these guidelines to estimate a Solvent Angle:

For a good signal-to-noise ratio, use the most transparent mobile phase available for your wavelength range (for example, acetonitrile instead of methanol, for low UV absorbance).

Inject amounts of sample that produce peak heights of less than 1 AU at the highest absorbance. This condition produces the best Purity Angle determinations. If necessary, use the MaxPlot derived channel function to extract this data channel.

To estimate solvent angle when using Noise+Solvent for the Threshold Criteria parameter:

1. Inject six replicates of a chemically pure standard at the highest concentration that is to be routinely used.

   Recommendation: Ensure that this concentration results in a peak height of less than 1 AU at the maximum absorbance.

2. Create a spectral library from one of the standard injections.

3. Create a PDA processing method to perform Peak Purity testing and Library Matching.

4. Set the Threshold Criteria to Noise.

5. Save the processing method in a method set, and then apply the method set.

6. Record the Purity Angle and Match Angle calculated for each injection.
7. Estimate the Solvent Angle based on the Purity or Match Angles you obtain from the replicate standard injections.

Tip: For best results, the Solvent Angle should be experimentally determined for each method using a chemically pure reference standard.

8. For the Purity Angle, specify a Solvent Angle equal to the highest Purity Angle obtained.

Tip: This Solvent Angle can be used for all sample concentrations less than the highest concentration. The Purity Threshold computed for each analysis is the sum of the Solvent Angle and the Noise Angle.

9. For the Library Match Angle, specify a Solvent Angle equal to the highest Match Angle obtained.

Tip: This Solvent Angle can be used for all sample concentrations less than the highest concentration. The Match Threshold computed for each analysis is the sum of the Solvent Angle and the Noise Angle.

For a properly set Solvent Angle

A Purity Angle greater than the Purity Threshold is evidence that the unknown contains a co-elutant. Conversely, a Purity Angle less than the Purity Threshold indicates that there is no evidence of co-elution.

A Match Angle greater than the Match Threshold is evidence that either the unknown spectrum comes from a compound that is different from the compound from which the library spectra were derived, or the unknown is a mixture or it is not the same component that is in the library. Conversely, a Match Angle less than the Match Threshold indicates that the unknown spectrum is very similar to the matching spectrum from the library.

Threshold criteria considerations

Use the Threshold Criteria parameter to choose a method for calculating the non-ideal contributions that affect Peak Purity testing and Library Matching. To compensate for the effect of noise or solvent contribution during Peak Purity testing and Library Matching, you can set the Threshold Criteria parameter in the Purity tab and in the PDA Library Search tab of the PDA Processing Method window.

When you interpret Peak Purity and Library Matching results, compare the Purity or Match Angle to the Purity or Match Threshold (Angle) to determine if a peak is spectrally homogeneous, or whether spectra are similar or different.

Tip: For Peak Purity testing, use AutoThreshold whenever possible.

For Peak Purity testing, setting the Threshold Criteria too low may cause false positive results for co-elution. Setting the Threshold Criteria too high may cause you to miss evidence of co-elution.

If you select AutoThreshold (the default) for the Threshold Criteria parameter in the Purity tab of the Processing Method window, Empower software computes the Threshold as the sum of the Noise Angle and the Solvent Angle. You must select an appropriate noise interval to allow Empower to compute the Noise Angle. Empower calculates the Solvent Angle for you.

If you select Noise+Solvent for the Threshold Criteria parameter in the Purity tab or in the PDA Library Search tab of the
Processing Method window, Empower software again computes the Threshold as the sum of the Noise Angle and the Solvent Angle. You still need to select an appropriate noise interval to allow Empower to compute the Noise Angle. However, you must determine the Solvent Angle manually.

**Using AutoThreshold as the threshold criteria**

Empower software uses the Threshold Criteria to compute the Threshold Angle used in Peak Purity testing and Multicomponent Peak Purity testing. When used in Peak Purity testing and Multicomponent Peak Purity testing, the default value for Threshold Criteria is AutoThreshold. Because AutoThreshold automatically calculates the Solvent Angle, selecting AutoThreshold disables the Solvent Angle parameter in the Purity tab.

When you perform Peak Purity testing or Multicomponent Peak Purity testing using a PDA processing method with AutoThreshold selected, Empower software automatically performs the following steps:

- Determines the Solvent Angle for each peak in a chromatogram. The Solvent Angle is automatically calculated as a function of the maximum spectral absorbance (MSA) of a peak. MSA is the maximum value of baseline-corrected spectral absorbance for a peak.
- Calculates the Noise Angle based on the noise interval you selected and the peak spectra.
- Computes the Threshold Angle for each peak in the chromatogram as the sum of its Noise and Solvent Angles.

AutoThreshold accurately calculates a Solvent Angle for each peak, but you still need to validate AutoThreshold for a particular separation.

To validate AutoThreshold for your application:

1. Make six replicate injections of a compound (standard) that is known to yield a baseline-resolved and pure peak.
2. Record the maximum spectral absorbance (MSA) of this standard.
   
   Tip: To use AutoThreshold, Waters recommends that the MSA of the standard be less than 1 AU. Since photometric error increases above 1 AU, Peak Purity measurements become significantly less sensitive to the presence of co-elution when the MSA of a compound exceeds 1 AU.
3. Determine the noise interval.
4. In the Processing Method window, on the Purity tab, confirm that AutoThreshold is selected.
5. Modify other parameters as appropriate, and then save the method.
6. Process the PDA data.
7. For each injection, ensure that the Purity Angle of the pure peak is less than the Threshold Angle.

If the Purity Angle is less than the Threshold Angle in all six injections, you can use AutoThreshold to perform Peak Purity testing on your unknowns. You can use the Threshold value obtained from AutoThreshold for Peak Purity testing.
on any unknown as long as its MSA is less than 1.0 AU and less than five times the MSA of the standard.

For example:

If the MSA of the standard is 0.1 AU, you can use AutoThreshold for unknowns with MSAs up to 0.5 AU.

If the MSA of the standard is 0.2 AU, you can use AutoThreshold for unknowns with MSAs up to 1.0 AU.

If the MSA of the standard is 0.5 AU, you can use AutoThreshold for unknowns with MSAs up to 1.0 AU.

If the Purity Angle is greater than the Threshold Angle, then Waters recommends that you do not use AutoThreshold. In this case:

Use the Noise+Solvent value for the Threshold Criteria parameter in the PDA processing method.

Manually set the Solvent Angle.

**ADDITIONAL INFORMATION**

Auto Threshold cannot be used for peaks greater than 1 AU.